EFFECTS OF CORN SILAGE INOCULANTS ON AEROBIC STABILITY

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ABSTRACT. *Aerobic stability of corn silage can be a major problem for farmers, particularly in warm weather. Silage inoculants, while the most common type of silage additive, have not been consistently effective at improving aerobic stability. This study investigated the efficacy of new inoculant products on corn silage in mini−silos over three trials (one per year). Two new approaches were tested: an enhanced homofermentative inoculant, and heterofermentative lactic acid bacteria (*Lactobacillus buchneri*). These approaches were compared with untreated as well as three standard homofermentative lactic acid bacterial inoculants. The standard inoculants on average reduced aerobic stability 12 h relative to untreated silage. The enhanced inoculant increased stability in one of three trials. The* L. buchneri *inoculants improved stability consistently in all three trials except in one case where one of these products had low viability. Of the new inoculants, the* L. buchneri *products appear to be most consistent at improving the aerobic stability of corn silage.*

Keywords. *Aerobic stability, Corn, Inoculant, Lactic acid bacteria, Silage.*

Inoculants are the most common additives used in making silage in the U.S. These products have selected homofermentative lactic acid bacteria to supplement the natural population of lactic acid bacteria on the crop and ensure a rapid silage fermentation that is higher in lactic acid and lower in acetic acid and ethanol. While these bacteria have provided improvements in dry matter (DM) recovery and animal performance, aerobic stability (the time until the silage begins to heat during feed out) has been decreased by inoculants approximately one−third of the time in reported research (Muck and Kung, 1997). The majority of the reductions in aerobic stability have occurred in corn and other whole−crop grain silages, where aerobic stability problems are common even with good silage management.

Inoculant manufacturers are aware of this problem and have been working on developing inoculants that more consistently improve aerobic stability. They have been investigating several approaches, two of which are available commercially now. The first is to use homofermentative lactic acid bacteria that can inhibit yeasts, the frequent initiators of spoilage and heating in silages at feeding (Woolford, 1990). Such bacteria would presumably work like current inoculants relative to fermentation and animal performance. The second approach is to inoculate the crop with *Lactobacillus buchneri*, a heterofermentative lactic acid bacterium. In this approach, acetic acid production should be increased. Because acetic acid is a better inhibitor of yeasts than lactic acid at a given pH (Moon, 1983), yeast counts should be lowered, improving stability.

The objective of this study was to test three standard homofermentative inoculants, one enhanced homofermentative inoculant, and three *L. buchneri* inoculants for their efficacy in improving the aerobic stability of corn silage as well as for their effects on fermentation and DM recovery.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Whole−crop corn was harvested with a forage harvester in each of three years. The chopped corn was ensiled in 35.5 × 10 cm dia. PVC silos sealed with a rubber end cap on one end and with black plastic secured with duct tape on the other. The black plastic and duct tape were used to create a seal that would allow some oxygen to enter the silos during storage, creating conditions more like field−scale silos. Depending on the year, 1.5 or 2 kg fresh crop were placed in each silo. Dry matter densities were 173, 246, and 263 kg/m^3 in 1999, 2000, and 2001, respectively. Four silos were ensiled per treatment. The number of treatments varied from year to year depending on product availability at the time of each trial, but most treatments were tested all three years. Treatments included an uninoculated control, three standard commercial corn silage inoculants (Standard A: *Pediococcus pentosaceus* and *Propionibacterium jenseni*, Standard B: *Lactobacillus plantarum* and *Enterococcus faecium*, and Standard C: *L. plantarum* and *E. faecium*), a new product with improved homofermentative strains (Enhanced A: two strains of *L. plantarum*), and three products with only the heterofermentative species *L. buchneri* (*L. buchneri* A and C were commercial products; *L. buchneri* B was our own strain, *L. buchneri* TY−16).

All products were applied at labeled rates (i.e., specified g original product/t crop); however, the amount of dilution with water was greater than specified in the products' directions. This was done so that all inoculant solutions were

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applied at the same rate (1 g/50 g crop). The control received 1 g water/50 g crop. The amount of chopped corn for a given silo was weighed out, sprayed with the appropriate inoculant solution using a plant sprayer (one sprayer for each treatment), mixed by hand, and then placed into the silo by hand with periodic tamping. Equipment coming in contact with treated corn was washed and then wiped with ethanol between treatments to prevent cross−contamination. Silos were weighed before and after filling to determine the actual amount ensiled. Over the course of filling the silos for all treatments, four samples of untreated chopped corn were taken for analysis of initial characteristics, and all inoculant solutions were analyzed for lactic acid bacteria counts.

The silos were opened after a minimum of 90 days ensiling. Silos were weighed prior to emptying. The spoiled silage on the top was removed and weighed. The rest of the silage was removed, mixed, and analyzed for microbial groups, pH, fermentation products, crude protein, moisture content, and ash. The remainder was placed in Styrofoam buckets. A type−T thermocouple was placed approximately 10 cm into the silage, and average silage temperatures were recorded hourly until heating occurred. Aerobic stability was defined as the time after opening for silage temperature to reach 2˚C above ambient.

ANALYSES

The untreated chopped corn at ensiling and the silages at silo opening were analyzed for the same constituents with the exception that fermentation products were determined only on silages. Duplicate samples were taken for moisture determination by freeze−drying. After moisture determination, the duplicate freeze−dried samples were ground together and analyzed for crude protein by a Leco FP−2000A nitrogen analyzer (Leco Corp., St. Joseph, Mich.) and ash at 550˚C for 3 h. Another portion of original sample (20 g) was diluted 10:1 with autoclaved distilled water and blended in a 0.5 L Waring commercial laboratory blender for 30 s. The diluted sample was enumerated immediately for acid−tolerant lactic acid bacteria (LAB) using Rogosa SL agar (Difco 0480, Becton Dickinson, Sparks, Md.), acetic acid bacteria (ethanol−yeast extract agar; Muck et al., 1992), *Bacillus*-spores (nutrient agar, Difco 0001; heat shock dilutions at 78˚C for 12 min), and yeasts and molds (malt agar, Difco 0024; acidified with lactic acid to pH 3.5). Plates were incubated aerobically at 30˚C for 72 h except for the ethanol−yeast extract agar (5 d) and Rogosa SL agar (anaerobic incubation). The remainder of the diluted sample was strained through cheesecloth and analyzed for pH and fermentation products (lactic, acetic, propionic, butyric, and succinic acids, ethanol, and 2−3 butanediol as per Muck and Dickerson, 1988). All inoculant solutions at ensiling were plated on Rogosa SL agar to measure the number of LAB in the inoculant solutions and determine actual LAB application rates.

Losses during ensiling were calculated by the difference in the DM removed from that ensiled. Total losses were calculated based on the weight of good silage DM removed and the weight of crop DM ensiled from each silo. Spoilage losses consisted of the moldy silage DM removed from the top of each silo. Gaseous losses were calculated by subtracting spoilage losses from total losses.

Significant differences ($P < 0.05$) in silage characteristics between inoculant treatments within a year were determined using the PROC GLM procedure of SAS (SAS Institute, Inc., Cary, N.C.).

Table 1. Initial characteristics of the chopped whole−plant corn ensiled in each year.

Characteristic	1999	2000	2001
Dry matter (g/kg)	327	458	367
pH	5.90	5.98	5.79
Crude protein (g/kg DM)	90	64	71
Lactic acid bacteria (log(cfu/g))	6.49	5.04	6.02
Yeast (log(cfu/g))	6.28	8.02	7.40
Mold (log(cfu/g))	4.49	6.62	6.17
Acetic acid bacteria (log(cfu/g))	6.48	6.86	6.83
Bacillus spores (log (cfu/g))	5.18	ND[a]	ND

[a] ND − not determined.

RESULTS

INITIAL CHARACTERISTICS

Characteristics of the corn at ensiling are shown in table 1. Corn in 2000 was more mature and drier than in the other two years. The year 2000 corn also had the lowest crude protein concentration and epiphytic lactic acid bacterial count, while having the highest numerical counts of spoilage microorganisms at ensiling.

The levels of LAB applied by the various treatments are given in table 2. In 1999, inoculant populations were either similar to or approximately one order of magnitude less than the epiphytic LAB population. The lone exception was the *L. buchneri* B treatment, which was 1.5 log(cfu/g fresh crop) less. In 2000, LAB application rates were similar to or higher than the epiphytic population with the exception of Standard C and *L. buchneri* B. The viability of these latter two were far less than anticipated for unknown reasons. In 2001, the inoculant LAB rates were more uniform and approximately 0.5 log units above or below the epiphytic population.

SILAGE CHARACTERISTICS

The aerobic stabilities of the silages in all three years are summarized in table 3. In 1999, the stability of the corn silage treated with Standard A was numerically greater than the untreated corn silage, whereas the other two standard inoculants produced less stable silages. The Enhanced A was numerically less than the untreated silage as well. However, none of these differences were statistically significant ($P = 0.05$). The *L. buchneri* treatments significantly improved aerobic stability ($P < 0.05$).

In 2000, only the *L. buchneri* A treatment significantly improved aerobic stability compared with the untreated control. All other treated silages were not statistically different from the untreated silage.

Table 2. Lactic acid bacteria applied [log(cfu/g crop)] by treatments in each year.

Treatment	1999	2000	2001
Standard A (*P. pentosaceus*, *Pr. jenseni*)	5.26	5.00	5.87
Standard B (*L. plantarum*, *E. faecium*)	5.44	5.08	5.84
Standard C (*L. plantarum*, *E. faecium*)	6.43	2.63	5.80
Enhanced A (*L. plantarum*)	5.88	5.63	6.01
L. buchneri A	6.35	6.10	6.59
L. buchneri B	4.93	3.05	−−
L. buchneri C	−−[a]	6.07	6.57

[a] Treatment was not done in that year.

Table 3. Aerobic stabilities (h from silo opening until silage temperature reached 2˚C above ambient) of silages in all three years.

Treatment	1999	2000	2001
Untreated	75	97	75
Standard A (*P. pentosaceus, Pr. jenseni*)	91	84	36
Standard B (*L. plantarum, E. faecium*)	71	77	69
Standard C (*L. plantarum, E. faecium*)	50	91	66
Enhanced A (*L. plantarum*)	51	70	104
L. buchneri A	217	197	886
L. buchneri B	178	96	––
L. buchneri C	––[a]	119	529
S.E.	17.0	18.8	9.7

[a] Treatment was not done in that year.

In 2001, the Standard A silage was significantly less stable than the untreated, whereas the Enhanced A and *L. buchneri* treated silages were all significantly more stable than the untreated.

The pH and the principal fermentation products are given in table 4. The pH values of untreated silages in all three years (3.64 to 3.87) were typical of corn silages observed in Wisconsin. Across the three years, no standard or enhanced inoculant treated silage had a pH that was significantly different from that of the corresponding untreated silage. The *L. buchneri* treated silages produced the only significant differences. These silages were typically 0.1 to 0.3 pH units higher than the pH values of untreated silages.

The fermentation product concentrations in the standard and enhanced inoculant treatment silages were generally similar to those of the untreated silages (table 4). The exception was the significantly elevated ethanol concentration in Standard A in 2001.

The *L. buchneri* treatments were more heterofermentative than the other treatments. The *L. buchneri* A and C inoculants consistently produced silages of lower lactic acid and higher acetic acid concentrations than those of the untreated silages. There were trends for higher ethanol, and in 2001 propionic acid was also measured in these treatments at 1.1 and 0.2 g/kg DM, respectively. The *L. buchneri* B in 1999 only had a trend toward lower lactic and higher acetic acid relative to the untreated, but it had a significantly higher ethanol concentration than the untreated silage. In 2000, the fermentation products from *L. buchneri* B were not different from that observed in the untreated.

The fungal characteristics of the silages at opening for the three years are shown in table 5. In 1999, there were no significant trends in microbial characteristics except for the yeasts and molds. Yeast counts in the *L. buchneri* treatments were reduced compared to those in the untreated, whereas

Table 4. The pH and fermentation characteristics (g/kg DM) of silages at opening.

Treatment	pH	Lactic Acid	Acetic Acid	Ethanol
1999				
Untreated	3.82	55	23	9
Standard A[a]	3.85	52	22	7
Standard B[b]	3.84	52	24	9
Standard C[c]	3.83	60	26	12
Enhanced A[d]	3.81	55	21	7
L. buchneri A	4.01	44	38	11
L. buchneri B	3.90	52	26	31
S.E.	0.013	2.6	2.0	2.5
2000				
Untreated	3.87	53	10	4
Standard A	3.89	58	11	5
Standard B	3.90	55	11	5
Standard C	3.90	59	11	5
Enhanced A	3.90	59	11	6
L. buchneri A	4.11	36	24	8
L. buchneri B	3.90	60	11	5
L. buchneri C	4.06	42	23	8
S.E.	0.024	1.9	0.6	0.7
2001				
Untreated	3.64	73	18	9
Standard A	3.71	89	23	20
Standard B	3.65	81	20	13
Standard C	3.62	75	16	10
Enhanced A	3.64	82	18	9
L. buchneri A	4.01	38	70	11
L. buchneri C	3.84	65	55	12
S.E.	0.018	8.1	3.2	2.0

[a] Standard A =*P. pentosaceus* and *Pr. jenseni.*
[b] Standard B = *L. plantarum* and *E. faecium.*
[c] Standard C = *L. plantarum* and *E. faecium.*
[d] Enhanced A = *L. plantarum.*

Standard C and Enhanced A had elevated yeast counts. Mold counts were generally similar to yeast counts within a treatment. The average levels of acetic acid bacteria and *Bacillus* spores across all treatments were 4.5 and 2.7 log(cfu/g), respectively.

In 2000, there were no statistically significant differences among treatments for molds. The *L. buchneri* A treatment had the lowest yeast count, which was significantly different ($P < 0.05$) from those of the standard and enhanced inoculants but not from that of the untreated control. There were no trends relative to acetic acid bacteria and *Bacillus* spores except that Enhanced A had a higher acetic acid bacterial

Table 5. Fungal characteristics [log(cfu/g)] of silages at opening in all three years.

	1999		2000		2001	
Treatment	Yeasts	Molds	Yeasts	Molds	Yeasts	Molds
Untreated	3.15	3.80	3.42	3.23	3.63	2.57
Standard A (*P. pentosaceus, Pr. jenseni*)	2.90	2.77	3.77	3.56	5.43	2.66
Standard B (*L. plantarum, E. faecium*)	3.27	2.62	3.87	3.15	3.08	1.87
Standard C (*L. plantarum, E. faecium*)	4.24	4.07	3.92	3.61	3.06	2.16
Enhanced A (*L. plantarum*)	4.13	4.04	3.75	3.76	2.73	2.18
L. buchneri A	1.00[a]	1.99	2.65	2.89	1.54	2.71
L. buchneri B	1.55	1.18	3.74	3.74	––	––
L. buchneri C	––	––	2.93	2.89	1.00	1.77
S.E.	0.38	0.58	0.30	0.31	0.40	0.53

[a] The detectable limit was 2 log(cfu/g); samples below detectable level were set to 1 log(cfu/g).

population [5.4 log(cfu/g)] than those of the other treatments [average of 4.6 log(cfu/g)].

In 2001, Standard A had higher yeast counts than the untreated silages, whereas the Enhanced A and the *L. buchneri* treatments had lower yeast counts than the untreated silages. Standard A and *L. buchneri* C had elevated acetic acid bacterial counts [6.4 and 6.0 log(cfu/g)] compared to the average of the other treatments [4.8 log(cfu/g)].

Dry Matter Losses

The average DM losses from the silos for all three years are given in table 6. Losses in all three years were higher than normally seen in laboratory silos. This was by design because of the leaky seals at the top of the silos created by the duct tape and plastic. The highest losses occurred in 1999, the year when density was lowest.

There was considerable variability among replicates and thus no significant differences within a year. The standard inoculants tended to have reduced gaseous (1999) and spoilage (1999, 2001) losses than the untreated, leading to trends for reduced total losses in both years. The Enhanced A treatment had the numerically lowest losses in all three categories in 1999, but the trends for this treatment in 2000 and 2001 were for numerically higher losses than the untreated. Numerically, the *L. buchneri* treatments generally ranked as having some of the highest gaseous and spoilage losses in 1999 and 2001, whereas in 2000 their losses were indistinguishable from those of the other treatments.

Table 6. Dry matter losses in silages.

Treatment	Gaseous Loss (%)	Spoilage Loss (%)	Total Losses (%)
1999			
Untreated	10.3	22.5	32.8
Standard A[a]	7.6	21.9	29.4
Standard B[b]	8.7	18.0	26.7
Standard C[c]	9.1	16.7	25.8
Enhanced A[d]	7.0	17.5	24.5
L. buchneri A	10.5	19.1	29.5
L. buchneri B	9.7	22.7	32.4
S.E.	1.00	2.31	2.72
2000			
Untreated	11.4	4.7	16.2
Standard A	13.6	6.3	19.8
Standard B	13.3	5.1	18.4
Standard C	12.0	4.5	16.5
Enhanced A	14.5	6.4	20.9
L. buchneri A	13.5	4.6	18.1
L. buchneri B	14.8	4.7	19.6
L. buchneri C	12.1	5.0	17.1
S.E.	1.00	0.70	1.19
2001			
Untreated	8.1	6.3	14.3
Standard A	9.5	4.2	13.7
Standard B	9.1	2.9	12.0
Standard C	7.9	5.9	13.8
Enhanced A	8.7	7.9	16.6
L. buchneri A	9.8	7.4	17.2
L. buchneri C	13.6	6.9	20.5
S.E.	1.06	2.08	2.65

[a] Standard A =*P. pentosaceus* and *Pr. jenseni*.
[b] Standard B = *L. plantarum* and *E. faecium*.
[c] Standard C = *L. plantarum* and *E. faecium*.
[d] Enhanced A = *L. plantarum*.

Discussion

Standard Inoculants

An extensive survey of inoculant studies found that approximately one−third of studies reported that homofermentative LAB inoculants reduced aerobic stability and that most of these reductions occurred in whole−crop corn and small grain silages (Muck and Kung, 1997). They speculated that this occurred because a reduction in pH by inoculation was more difficult to achieve in such silages; they are typically low in pH, plus the inoculant bacteria would shift fermentation toward lactic acid and away from acetic acid. Because acetic acid is a better inhibitor of yeasts than lactic acid at a given pH (Moon, 1983), the shift in fermentation by such inoculants should be detrimental to aerobic stability, especially because yeasts are the most frequent initiators of heating in silages (Woolford, 1990).

In the present study with three standard inoculants (nine comparisons), only once was there a positive trend (Standard A in 1999) in aerobic stability by comparison with that of the untreated. The rest were negative, one of which was significantly worse (Standard A in 2001). On average, the standard inoculants reduced aerobic stability 12 h. This is a relatively small reduction, but it could be important when feeding corn silage under warm conditions.

The reason for this reduced stability appears to be linked with fermentation. There were some tendencies for lactic−to−acetic acid ratios to be increased by the standard inoculant. However, the most unstable standard inoculant treatment (Standard A in 2001) had the highest ethanol concentration across all treatments that year along with the highest yeast count, a possible cause of the ethanol (McDonald et al., 1991). This suggests that the more efficient fermentation of standard inoculants may create conditions that are more conducive to yeasts so that yeast populations are higher when the silo is opened and thus more susceptible to spoilage and heating. Overall, the results with the standard inoculants in the present study are in line with those observed in previous studies of inoculated corn silage.

Enhanced Inoculant

Only one enhanced homofermentative inoculant (Enhanced A) was tested. In general, Enhanced A behaved similarly to the standard inoculants. It tended to produce a more homofermentative silage compared to the untreated in each of the three years, although pH was not reduced. Consequently, from a pH and fermentation profile perspective, Enhanced A appeared similar to the standard inoculants. In two years, aerobic stability relative to the control was similar to that observed for the standard inoculants, an average reduction of 25 h. In one case, the yeast population relative to the untreated was high; in the other, acetic acid bacterial counts were elevated relative to the untreated. In 2001, however, aerobic stability was improved significantly (29 h) relative to the untreated silage. Overall, Enhanced A works from a fermentation perspective like a standard inoculant while, at least in one instance, improving aerobic stability. Certainly three trials in small−scale silos are insufficient to know how frequently and under what conditions this product will improve stability.

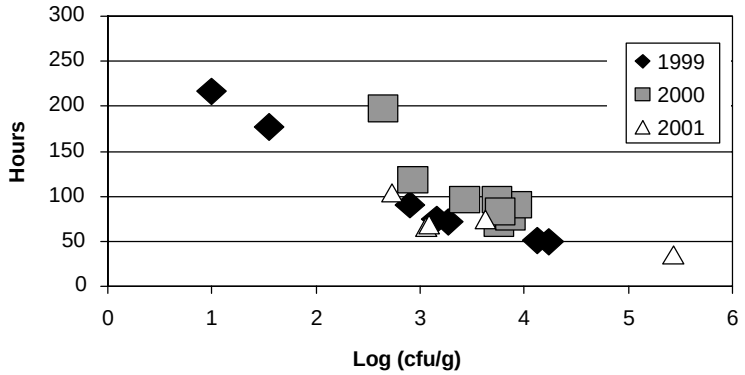

Figure 1. Aerobic stability (h from silo opening until silage temperature reached 2˚C above ambient) for all treatments as correlated with yeast count at silo opening.

LACTOBACILLUS BUCHNERI

Lactobacillus buchneri departs substantially from other silage inoculants because it is a heterofermentative LAB. This inoculant was only approved by the FDA in 2001 as a silage additive and thus is only in its third year in the U.S. market. This species has been tested relative to aerobic stability in a variety of crops: corn (Driehuis et al., 1999a, 1999b; Ranjit and Kung, 2000; Ranjit et al., 2002), grass (Driehuis et al., 2001), alfalfa (Kung et al., 2003), whole−plant wheat (Weinberg et al., 1999), whole−plant barley (Kung and Ranjit, 2001; Taylor et al., 2002), high−moisture shelled corn (Kendall et al., 2002), and sorghum (Weinberg et al., 1999). All of these studies used the bacterial strain in the *L. buchneri* A treatment of the present study. This strain has shown itself to be remarkably robust and consistent in improving aerobic stability across a range of ensiled crops. The level of improvement in aerobic stability has been variable but consistently positive from less than a day to weeks greater than that of the untreated control. Similar to these other studies, *L. buchneri* A improved stability in all three years of the present study by 100 to 811 h relative to untreated silage.

The other two *L. buchneri* strains were also effective when applied at sufficient levels. *L. buchneri* B failed to improve stability when applied at 2 log(cfu/g) less than the epiphytic LAB population, but increased stability 103 h when applied at 1.5 log units below epiphytic levels. *L. buchneri* C increased stability 22 and 454 h in 2000 and 2001, respectively. Thus, the other *L. buchneri* strains tested here were effective on corn silage, although their increases in aerobic stability were somewhat less than that provided by *L. buchneri* A.

The effectiveness of *L. buchneri* appears to be due to a reduction in yeast populations. As shown in figure 1, stability across all treatments and years was highly correlated with yeast counts, and the *L. buchneri* treatments were the most effective at reducing yeast counts (table 5). At least in part, this may be due to the conversion of lactic to acetic acid by *L. buchneri* (Oude Elferink et al., 2001), considering that acetic acid is a better inhibitor of yeasts than lactic acid (Moon, 1983). However, the increased stability by *L. buchneri* B in 1999 in the absence of significant shifts in fermentation products and the very long stabilities by *L. buchneri* A and C in 2001 suggest that other mechanisms may also be involved. Both of these latter cases contained small amounts of propionic acid, which would contribute to stability but not to the extent observed.

Overall, the current study together with research by others suggests that *L. buchneri* is a consistent performer in terms of improving aerobic stability. This inoculant provides a good alternative for farmers wishing to improve the aerobic stability of corn silage at a lower cost than propionic acid and with a greater ease and level of safety than afforded by acids or anhydrous ammonia.

The major concern with *L. buchneri* may be how livestock will perform with high levels of acetic acid in the silage. Research is just beginning to be reported in this regard (Driehuis et al., 1999b; Kendall et al., 2002; Kung et al., 2003; Ranjit et al., 2002; Taylor et al., 2002). No reduction in intake has been observed, and milk production has generally been similar to that obtained feeding untreated silages. However, more animal performance research is needed where the untreated silage is unstable in order to better gauge the economic value of *L. buchneri*.

CONCLUSIONS

Of the new corn silage inoculants available to farmers, the *L. buchneri* inoculants provided the most consistent improvement in aerobic stability. An enhanced standard inoculant was better than standard inoculants in one year of three relative to aerobic stability. At this stage of testing, selection of a corn silage inoculant appears to hinge on the most important goal(s) of the farmer. If poor aerobic stability in corn silage and its effect on animal performance are consistent problems that cannot be solved by improved silo management, then the *L. buchneri* products show the most promise. However, if the primary goals are improved animal performance and improved dry matter recovery, then the enhanced standard and conventional homofermentative inoculants appear more likely to achieve success.

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